

SUBJECT:	Techniques in Histopathology 560055
ECTS :	2,5
COORDINATORS:	José García Valero Gustavo Egea Guri
STAFF:	Mercè Durfort i Coll (<i>Fac. Biologia, Universitat de Barcelona</i>) Gustavo Egea Guri (<i>Fac. Medicina, Universitat de Barcelona</i>) [COORDINATOR] José Garcia Valero (<i>Fac. Biologia, Universitat de Barcelona</i>) [COORDINATOR] Joaquim Majó Masferrer (<i>Fac. Medicina, UAB. Hospitals de la Vall d'Hebron</i>) Juan Francisco Montes Castillo (<i>Fac. Biologia, Universitat de Barcelona</i>) José Francisco Ramírez Ruz (<i>Fac. Medicina, Universitat de Barcelona i Servei d'Anatomia Patològica, Hospital Clínic i Provincial de Barcelona</i>)

SUBJECT SCOPE AND OBJECTIVES

Subject scope

The progress in basic biomedical knowledge is having immediate consequences on the characterization of the dysfunctions at the molecular, cellular and tissue levels. This results in the design of new molecules and the improvement of both the experimental protocols and the progress in the scientific instruments used in research and diagnosis.

This subject aims to provide Biomedicine postgraduate students with the knowledge necessary for the professional practice of Medicine and Pharmacy (pathology, clinical analyses, diagnostic methods and therapeutic strategies, among others), as in basic research (Ph.D. studies, for example) and applied research (pharmaceutical industries, clinical research, etc)

Learning objectives

At the end of the course, the student will achieve the following learning outcomes:

- a. To acquire the conceptual basis of techniques used in the study of histopathology.
- b. To assimilate the methodological strategies applied in characterizing the pathological patterns at molecular, cellular and tissue levels.
- c. To acquire the knowledge to study the pathological patterns by characterizing different *in vivo* and *in vitro* experimental models at molecular, cellular and tissue levels.
- d. To acquire technical knowledge about the equipment used in Histopathology (conceptual basis, application, operation and methodology to interpret the results).
- e. The main sources of histopathological information.

LECTURES

Lecture topics

Lectures (20 hours) are distributed among 10 h of basic topics and 2 h of seminars given by specialists in histopathological methodologies.

1. Sampling processing

Characteristics of biological samples. Methods for obtaining samples. Sample stabilization: fixation, freezing, inclusion, encasing, tissue microarrays. Methods of preparations: sectioning, homogenization, matrices of cellular growth.

2. Topographic staining

Staining strategies. Optical differentiation of cell and tissue compartments. Applications.

3. Histochemical techniques

Cell and tissue targets. Processing of samples. Methods used for connective tissue, amyloid and fibrin. Histochemistry of sugars, proteins and lipids. Detection of microorganisms. Histochemistry of pigments and minerals. Detection of neurosecretory granules.

4. Methods for studying systems and organs

Nervous system. Cardiovascular and respiratory systems. Urogenital system. Endocrine system. Digestive system. Skin. Motor system. Hematopoietic and lymph systems.

5. Immunohistochemical and histochemical techniques

Antigens and antibodies. Standardization of protocols. Quality control. Preparation of samples. Pre- and post- embedding techniques. Direct and indirect procedures. Labeling: fluorochromes, enzymes, electron-dense stains, biotin. Autofluorescence. Spectra of fluorescent markers. Microscopes: light microscope, epifluorescent microscope, confocal microscope, transmission and scanning electron microscopes. Cytofluorometry. Most frequent pitfalls. Result analysis. Use of light and electron microscopy to study and to diagnose tumors. Immunohistochemical markers. Labeling with proteins of high affinity: lectins and enzymes.

6. PCR and Real Time PCR

DNA and RNA amplification techniques. Parameters affecting the implementation of PCR. PCR instruments. Results analysis. Technical bases of RT-PCR. Instruments, monitoring, sources of error and analysis of results. Competitive RT-PCR. Quantitative RT-PCR. Semiquantitative RT-PCR.

7. *In situ* hybridization techniques

Fundamentals of hybridization. Sample processing. Construction, use and detection of probes. Sources of error and analysis of results. *In situ* hybridization techniques (ISH): probes, protocols, instruments and results. Fluorescence *in situ* hybridization techniques (FISH): probes, protocols, instruments and results. *In situ* PCR techniques: probes, protocols, instruments and results. Data Banks and available software.

8. Microarrays

Microarray fundamentals. Gene expression microarrays: construction, protocols, instruments and results. Protein expression microarrays: construction, protocols, instruments and results.

9. Laser microdissection

Fundamentals of the technique. Instrument types. Sample obtaining. Protocols to extract protein and nucleic acids. Limits and advantages of the technique.

10. Image Analysis. Basic equipment used in Histology and Anatomical Pathology

PROGRAM OF SEMINARS

Seminar #1. To be announced.

Seminar #2. To be announced

PRACTICAL SESSIONS

Each student will do two practical sessions totalling 10 hours.

Session #1. Tissue processing and sampling. Topographic stain applied to connective tissue. Specific staining of elastin (histopathologic diagnostics of Marfan syndrome).

Session #2. Immunohistochemical diagnostics: use of mono- and polyclonal antibodies and labelling (fluorochromes and HRP).

TEACHING METHODOLOGY AND ORGANIZATION

Lectures

Theoretical lectures. These will last 50 min approximately with the technical support of PC and video.

Practical lectures. These will be given in the laboratory #22 of the faculty of Biology. This lab is supplied with all the necessary material (reagents and small equipment).

Personal homework

It is also necessary to carry out a personal homework under the supervision of the coordinators. This work will be composed of the following:

- a. The students should resolve a practical case. There will be teams composed by 2-4 students.
- b. There will also be a part of bibliographical search of some specific item indicated in the programme of the course. This work will be performed for each student.

Study activities

Each student should dedicate enough time to prepare and to enhance the content given in the lecture sessions. The course is divided in several classes, whose content will be detailed in the Campus Virtual. At the same time, the student should prepare strategies and study methodologies that help him to get the aims of the subject.

Tutorials

The tutors will attend whichever request related with the subject made by the students. This could be done personally (according to the availability of the different teachers) or through the forum opens in the Campus Virtual.

EVALUATION

Both the theoretical and practical skills obtained by the student will be evaluated. The evaluation will also take into account the homework performed by the student.

The final score will be the following:

a) 60% of the final score will be obtained after carrying out a test. It will be composed of 25 questions with 4 possible answers, from which only one is correct. To pass the exam it is necessary to obtain a minimum of 15 out of 25 correct answers. There is no negative score if the question is not or incorrectly answered.

b) 40% of the final score will be obtained after evaluation of the student homework indicated above.

BIBLIOGRAPHY

There is not a unique bibliographical source that encloses all the different items of the subject. Therefore, it will be used different Cell Biology, Histology and pathology textbooks, as well as web pages, which altogether will be indicated at the end of each lesson.